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MERCURY'S INTERACTION WITH SELENIUM

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Project Description

Environmental mercury (Hg) can either bioaccumulate within an aquatic food chain as the highly toxic form methylmercury (MeHg) or biologically retire as inert forms such as mercury sulfide (HgS) and mercury selenide (HgSe). Based on ongoing CATM research, it appears that the high-affinity binding between Se and Hg is involved in numerous aspects of MeHg toxicity in the environment and in physiology. This project examines these interactions from multiple perspectives in order to assess the environmental benefits of Se binding with Hg and the physiological harms of Hg binding with Se.

Goals

In order to better understand how Se limits Hg accumulation and how Hg harms Se physiology, it is important to study their interactions from multiple perspectives in an integrated research program. Research in previous years has used animal models to study Se-dependent protection against Hg toxicity and invertebrates to assess Se-dependent Hg retirement from aquatic ecosystems. To continue with an integrated approach to analysis, the goals of this project are threefold: to assess *environmental* factors of Hg and Se accumulation by analyzing plants and invertebrates and the potential ways of remotely detecting Hg and Se accumulation in plants, to investigate the influence of Se status on MeHg toxicity at the *cellular level* in time–dose-dependent studies, and to investigate human effects of Hg accumulation through the examination of heart tissues of human patients with dilated cardiomyopathy.

Rationale

The importance of the Se and Hg interaction has many environmental and phsyiological implications that can influence MeHg exposure risks. Se, an important nutrient present in many foods including fish, has a strong protective effect against Hg toxicity because of its exceptional binding affinity for Hg. However, a different perspective on this interaction suggests MeHg toxicity is actually the result of this binding affinity whereby Hg sequesters intracellular inorganic Se and, thereby, inhibits the formation of vital selenoenzymes in the central nervous system. In this regard, the greatest likelihood of risk to MeHg-exposed populations will occur in Se-poor areas whose diets originate from locally grown foods and freshwater fish. Se-poor regions of the world include much of northern Europe, most of Africa, New Zealand, and many parts of Asia. If their diets comprise foods grown in the local area, their low Se status will offer less Se-dependent protection against MeHg exposure. Likewise, lakes with low Se availability present the greatest harm, since lakes with low Se have displayed increased MeHg bioaccumulation in fish (1–6).

Se availability for uptake by plant life in the aquatic environment is likely to have an important effect on Hg bioaccumulation in the food chain. Formation of HgSe is minimal in the water column but greatly influenced by the molecular species of Hg and Se that occur within living cells. Mercury hyperaccumulator plant species and invertebrates are likely to contribute to Hg bioaccumulation and retirement through HgSe formation as a result of biochemical processes that parallel those occurring in animal cells. Since HgSe is poorly soluble and resistant to dissociation, it is unlikely to be absorbed. Therefore, if Hg accumulation is reduced in the insects that make up 90% of the food consumed by fish, it will influence the amount of Hg accumulation in fish.

Currently, there are no techniques available that allow a precise, rapid, and inexpensive means of identifying areas that have high concentrations of heavy metals. Extensive sampling is needed to map soil concentrations. However, spectroscopic analysis of vegetation may provide a method for regional detection. Remote sensing has shown that Se-rich soybeans showed a spectral reflectance shift to shorter wavelengths in the long-wavelength edge of the chlorophyll absorption band centered at 680 nm and higher reflectance in the 550–650-nm region. Therefore, Se incorporation in plants causes a specific shift in reflectance of light at 660 nm. This spectral shift may enable rapid and remote detection of Se distribution, thereby establishing a means of identifying areas of high vs. low Se soil availability. Although not yet assessed, if this approach proves to be sufficiently sensitive, sensing through satellite imaging may eventually become possible. This method could then be used to enable global detection of Se-poor regions with greater risks of Hg exposure.

Studies performed in eastern Finland suggest increased Hg exposure from eating lake fish is associated with increased cardiovascular risk and unusually high concentrations of Hg and antimony (Sb) accumulation were reported in heart tissues of human patients suffering from dilated cardiomyopathy (DCM). These levels have not been confirmed and may be due to analytical errors resulting from the small sample sizes obtained using biopsy methods. However, if validated, this phenomenon could lead to further directions in understanding MeHg-associated cardiovascular disease. Since almost half of all heart transplants are performed on DCM patients, significant amounts of heart tissue can be accessed from the explanted heart removed during transplantation. The larger tissue samples will provide an opportunity for the highest-quality analytical evaluations so that these levels can be reassessed and validated. Likewise, Se levels will also be assessed, since Se has an important role in Hg toxicity, and Hg accumulation seldom occurs without the formation of HgSe. The influence of Se on Hg demethylation rates and loss of available Se for sustaining selenoenzyme activities are also important metabolic interactions that need to be assessed for understanding these types of pathologies. The entry of inorganic Hg into the brain is largely prevented by the blood–brain barrier, whereas organic Hg can pass the barrier more easily (7). However, it is interesting to note that a significant fraction of the total Hg bound in animal and human brains (8–10) is inorganic, a portion of which appears to originate from MeHg demethylation (11). In the brain, glial cells appear to be responsible for this demethylation activity (12). MeHg at a concentration of 0.1 µm has shown to be lethal in cell cultures (13), but no one has measured the direct effects of MeHg exposure on Se-dependent enzyme activities in cultured cells.

Approach

Mercury–Selenium Interactions in Plants and Invertebrates

Formation of HgSe will be investigated in plants known to hyperaccumulate Hg and Se and also in a model invertebrate species (Achaeta domesticus; house crickets). Sodium selenite is the most commonly used form in Se nutrition studies, but the Se naturally present in foods is a mixture of organic forms including selenomethionine and selenocysteine. Therefore, these studies will compare the effectiveness of organic and inorganic forms of Se in protecting against MeHg toxicity. Since crickets are opportunistic cannibals, primary (initial torula yeast-based diets) and secondary (consumption of torula yeast-based diets supplemented with ground crickets from trophic level one) exposures are being investigated.

To investigate the spectral reflectance shift of vegetation induced by Se (and Hg binding of Se), rabbitfoot grass and water hyacinth are being grown under controlled conditions using hydroponic solutions. The plants are provided basal nutrient solutions with or without graduated concentrations of Hg, Se, or Hg and Se. As the plants grow, spectral reflectance of leaves is being measured by a spectrophotometer.

MeHg–Se Interactions at the Cellular Level

To understand the environmental and epidemiological effects of Hg exposure, it is important to understand Hg mechanisms at the cellular level, particularly in the central nervous system. The approach to this study is to use cell cultures grown in the presence of media containing graduated concentrations of Se and subject them to MeHg and inorganic Hg added at incremental log concentrations ranging from unexposed to toxic levels. Parallel studies of these Se-conditioned and Hg-exposed cultures will be challenged for specified time periods to establish the time- and dose-dependent effects of MeHg toxicity and Se's protective effects against toxicity. The end points measured include cell proliferation rates, viability, Hg and Se concentrations in the cells, Se-dependent enzyme activities, and rates of MeHg demethylation. Additionally, as a preliminary study to assess neuronal damage at the cellular level, apoptotic markers are being assessed using brains from previous animal studies. In these prior studies, rats were fed diets that were Se-deficient (0.1 μmol/kg), Se-adequate (1.0 μm/kg), or Se-enriched (10 μmol/kg) and supplemented with 50 μmol/kg) MeHgCl. Subsets of brains from each group were cleansed with buffered saline to remove all residual blood, frozen, and stored for future analysis.

Mercury's Effects on Cardiovascular Disease

Working with researchers and surgeons at the Mayo Clinic in Rochester, Minnesota, DCM heart transplant tissues and control autopsy heart tissues will be acquired and analyzed for Hg, Sb, and Se levels. The data will be compiled and statistically compared in order to confirm or refute the reported observations of unusually high Hg levels in DCM hearts.

Progress/Status

Experiments where graduated concentrations of Hg and Se were added to Hg accumulator plants have been conducted and spectral reflectance measurements made. Plant samples are currently being analyzed to assess Se-dependent effects on Hg accumulation in plants.

Experiments establishing Se-dependent protective effects against Hg toxicity have been conducted in crickets. The study of Hg bioavailability from tissues of crickets that had been fed equivalent amounts of Hg in the presence of varied amounts of Se are currently in the second phase of the artificial food web.

Cell culture lines are currently being established, and the specific culture studies are expected to be completed within the next few months.

A Synergy II plate reader for enzyme analysis has been purchased and installed, and a training workshop for this instrument has been conducted. Once the cell line parameters for this study are under way, GPx activity will be assessed using this plate reader.

The preliminary studies assessing markers of apoptosis in the animal brains is near completion.

Heart tissues from DCM patients are currently being collected and will be analyzed once the cohort of diseased and control samples is complete.

Quality Objectives Measurement/Data Acquisition

The quality objectives of this project are to obtain statistically valid and physiologically meaningful results regarding the interactions of mercury and selenium in plants and invertebrates. To measure, contrast, and compare the weight gains and survival of crickets provides a measure of their concentration-dependent effects.

Repeated cell culture studies are being performed to evaluate the effects of Hg exposure. At indicated times, the parameters are measured and the concentrations and time-dependent protective effects of Se against Hg toxicity are evaluated and individually plotted. Multiple independent assessments are performed, to determine the mean values and standard deviations for the survival and propagation rates. Selenoenzyme activity in cultured cells is assessed using established kit methods and their accompanying pool standards and controls.

In the study of elemental distributions in human heart samples, the quality objective is to obtain analytically accurate and precise determinations of elemental contents. Trace elements will be determined in batches that include certified quality control (QC) samples analyzed relative to calibration standards according to established protocol.

Assessment and Validation

The analysis data for the standard protocols used in this project indicate acceptable analytical accuracy and precision. Certified reference materials and control sample analytical results are within the expected rages. The sample analysis and numbers of samples used provide statistically meaning results, based on standard statistical analysis. The protocols have been established and are maintained to ensure accurate and precise analytical results are obtained; all sampling, instrument calibrations, and QC considerations are included in the protocols. QC samples including analytical blanks and certified reference materials will be included in each batch to ensure validity of observed analysis values.

Potential Applications and Benefits

The findings of these studies will provide important information for EPA, the U.S. Department of Energy, the U.S. Food and Drug Administration, and the World Health Organization. This information may assist these agencies in making regulatory policy decisions regarding Hg exposure. Assessing Se's involvement in Hg toxicity issues will help these agencies assess the risks of human Hg exposure and identify populations at risk and potential methods for protection and remediation.

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